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Chromium induced teratogenicity in female rat

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Abstract

Exposure to chromium (VI) (250, 500 and 750 ppm as potassium dichromate) via drinking water pregestationally in rats revealed embryo- and fetotoxic effects in the form of a significant reduction in the number of implantations and number of fetuses. An increase in the number of resorptions, pre-implantation and post-implantation loss in chromium (VI)-treated mothers was also observed. No significant visceral abnormality was found. A significant increase in sub-dermal hemorrhagic patches on thoracic and abdominal areas was found. Skeletal abnormality in the form of reduced ossification in parietal, interparietal and caudal bones was found in the fetuses of chromium (VI)-treated mothers. Chromium levels in blood, placenta and fetuses were found to be significantly increased in the 500 ppm and 750 ppm dosed groups. The duration of estrus cycle was significantly altered after chromium (VI) exposure. This study suggests that chromium exposure in rat causes a lower degree of toxicity than in mice as observed in our earlier studies.

Keywords: Hexavalent chromium; Drinking water; Pregestational period; Teratogenicity; Rats

1. Introduction

Chromium, an essential element for biological systems is also used in metallurgical processes, chrome plating, pigment production, tanning, textile, ceramic, glass and photographic industries. High concentrations of chromium (40–50 000 ppm) have been reported in the effluents from these industries [1]. Besides exposure to industrial workers, the general population is also exposed to this metal as it contaminates surface and ground water, agricultural land and aquatic life [2,3]. High levels of chromium are reported to impair gestational development as evidenced by epidemiological studies in female workers exposed to this metal in the work environment [4]. Exposure to chromium (VI) resulted in complications during pregnancy and childbirth in the form of toxicosis and puerperal hemorrhages in women employees at a dichromate manufacturing factory [5]. Tipton [6] reported the transfer of chromium from the

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mother to the bones of the developing fetus in humans. Pribluda [7] reported that the chromium content of bones of pregnant rats decreases with advancing gestation. Such released chromium may reach the circulatory system and enter feto-placental tissues through the placental barrier.

Our earlier study [8] revealed developmental changes in mice after oral exposure to chromium (VI) pregestationally. However no study to date has been carried out to determine the effect of chromium in rats exposed pregestationally. A significant difference in the feto-placental barrier of the two species (mice and rats) was observed, with mouse fetoplacental unit allowing a greater inflow of chromium (VI) from maternal blood to the fetuses whereas in rats the feto-placental barrier, to a greater extent, restricted the inflow of orally administered chromium (VI) [9]. Therefore, the present study was carried out to determine the effect of chromium (VI) on embryo-fetal development in rats exposed orally during the pregestational period of development. In addition, we wanted to establish the relative species susceptibility and also determine the distribution of chromium (VI) in the maternal and feto-placental unit.

2. Materials and methods

Adult Swiss albino female rats (120 days old; body weight 175 ± 25 g) of proven fertility from the Industrial Toxicology Research Centre bred colony were taken, synchronised for cyclicity and were divided into four equal groups. Group I was given tap water (Chromium level < 0.001 ppm) and served as controls. The remaining groups (group II, III and IV) were given 250, 500 or 750 ppm chromium (VI) [as potassium dichromate; AR, 99.9% pure, Ranbaxy Laboratories Ltd., India], respectively, for 20 days [one folliculogenesis cycle [8]]. The dose was selected on the basis of our earlier studies [8,10,11]. After the completion of the treatment, they were kept for mating (1:1) with normal healthy adult males overnight. The presence of sperm in the vaginal smear was designated as day '0' of gestation. The animals were kept in plastic cages individually under standard animal care conditions.

They were provided with pellet feed (Cr level 1.45 μg/g; Lipton India Ltd.) and water ad libitum. The body weight and water intake were recorded daily. Mating and fertility indices were calculated from the formulae:

$$\text{Mating index (\%)} = \frac{\text{No. of females kept for mating} - \text{number of mated females}}{\text{No. of females kept for mating}}$$

$$\text{Fertility index (\%)} = \frac{\text{No. of females mated} - \text{No. of pregnant females}}{\text{No. of females mated}}$$

Cesarian sections were performed on day 19 of gestation in 10 animals from each group. Blood was withdrawn from the heart and kept at −20°C for chromium estimation. Ovaries were removed, the number of corpora lutea counted, and number of fetuses/litter, number of live/dead fetuses, crown-rump length, number of resorptions, weight of fetuses with their respective placentae were recorded. Pre- and post-implantation loss was calculated as described by Palmer et al. [12]. One fetus/litter with its placenta was kept at −20°C for chromium estimation. One-third of the remaining fetuses were fixed in Bouin's fluid for examining the visceral abnormalities [13]. The remainder of the fetuses from each group were first examined for gross external abnormalities and then were fixed in 95% ethanol, eviscerated and stained by the Alizarin red S method [14] for examining skeletal deformities [15].

2.1. Chromium estimation

Maternal blood was measured, placenta and fetuses were washed with saline, blotted dry and weighed, then digested in a $HNO_3/HClO_4$ (6:1) mixture until a white residue remained. This residue was dissolved in an appropriate amount of 0.1 N HNO_3 and chromium was estimated on a DC Plasma Emission Spectrophotometer (Beckman Spectrospan V). Blank and chromium-spiked samples were run and analyzed simultaneously [16,17].

Table 1
Chromium-induced embryo- and feto-toxicity in rats treated during pregestational period

Parameter	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Mating Index (%)	100	80	70	40
Fertility Index (%)	96	75	57	31
Weight gain in mothers (g)	70.50 ± 5.19	65.02 ± 3.17[a]	60.92 ± 2.13[ab]	55.5 ± 3.01[abc]
Number of corpora lutea	10.02 ± 0.91	9.81 ± 0.95	7.13 ± 0.61[ab]	4.43 ± 0.50[abc]
Number of implantations	9.51 ± 0.96	9.61 ± 0.83	5.91 ± 0.39[ab]	2.27 ± 0.36[abc]
Number of live fetuses	9.11 ± 0.87	8.29 ± 0.93[a]	4.12 ± 0.51[ab]	1.21 ± 0.13[abc]
Number of resorptions	0.40 ± 0.24	1.09 ± 0.34[a]	1.72 ± 0.23[a]	1.03 ± 0.29[a]
Pre-implantation loss	5.08 ± 0.65	2.03 ± 0.31	17.11 ± 2.13[ab]	48.75 ± 5.81[abc]
Post-implantation loss	4.20 ± 0.41	13.73 ± 1.57[a]	30.28 ± 4.19[ab]	46.69 ± 5.21[abc]
Fetal weight (g)	3.54 ± 0.41	3.46 ± 0.29	3.08 ± 0.37	2.53 ± 0.31
Placental weight (g)	0.67 ± 0.08	0.71 ± 0.09[a]	0.79 ± 0.19[a]	0.86 ± 0.12[a]
Crown-rump length (cm)	3.18 ± 0.19	3.01 ± 0.27	2.78 ± 0.31	2.61 ± 0.23

Value represents mean ± S.E. of 10 rats in each group.
The significance of the difference among various groups was evaluated by applying one-way ANOVA; Significance level: $p < 0.05$.
Comparison between two groups: [a]vs. control; [b]vs. 250 ppm; [c]vs. 500 ppm.

2.2. Study of estrus cycle

Vaginal smears from 10 rats from each group were taken, once every morning, promptly spread on a clean slide and fixed in a solution of ethyl ether and ethanol. After staining with H and E, the slides were studied microscopically for quantification of epithelial cells and the frequency of cornified cells was calculated as a percentage. The intervals in days between two successive peaks in the frequency of cornified cells was taken as the length of each individual estrus cycle [18]. The study was continued for 12 consecutive estrus cycles.

2.3. Statistical analysis

Overall significance of differences in mean values between control and treatment groups was tested using one way ANOVA. Prior to the analysis, normality assumption of the data and homogeneity of variance between the experimental groups was ascertained. The means of the experimental groups from the controls and between two treatments were compared separately using Dunett's post hoc test [19]. Significance of difference in incidence of gross and skeletal abnormalities between group III and IV was tested using Fisher's Exact Test as the expected cell frequencies were less than five.

3. Results

No notable changes in behavior or clinical signs were observed in control or in treated dams. No mortality was observed during the experimental period. Daily water consumption in groups I, II, III and IV was 28.05, 25.78, 24.41 and 20.37 ml/rat/day, respectively. Based on this water intake, the chromium level reaching the treated groups (II, III and IV) was 6.44, 12.20 and 15.28 mg/rat/day. As the dose was increased, the mating index was found to be increasingly reduced. A similar pattern was seen with the fertility index which was calculated from the mated females (Table 1). Mothers of group IV and III registered a reduction in gestational weight gain (55.5 ± 3.01 and 60.92 ± 2.13 g, respectively). However, when compared with group I (controls), group IV and group III gained 21% and 14% less weight, respectively.

The number of corpora lutea was reduced in group III and group IV when compared to the control group (Table 1). The number of implantations was also significantly reduced in group III and IV when compared to controls. The number of fetuses per litter was significantly reduced in groups III and IV when compared to the control and the 250 ppm group (group II). However, when group III and IV were compared they did

Table 2
Incidences of gross and skeletal abnormalities in the pups of chromium-treated rats during the pregestational period

Parameter	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Gross abnormalities				
Number of pups/litter observed	72/10	70/10	51/10	19/10
Drooping wrist	0	0	0	6/4 (32)
Sub-dermal hemorrhagic patches	0	0	8/6 (16)	8/4 (42)[a]
Kinky tail	0	0	0	8/6 (42) [a]
Short tail	0	0	4/4 (9)	10/4 (53)[a]
Skeletal abnormalities				
Number of pups/litter observed	48/10	45/10	34/10	19/10
Reduced parietal ossification	0	0	0	12/10 (63)[a]
Reduced inter-parietal ossification	0	0	0	10/10 (53)[a]
Reduced caudal ossification	6/4 (12)	8/5(18)	18/8 (53)[a]	18/10 (95)[a]

Gross and skeletal abnormalities are represented as number of abnormal pups/litter observed; percentage in parentheses calculated by the total number of pups observed.
Statistical significance evaluated by Fisher's Exact test; comparison between two groups: [a]vs. control. Significance level: $p < 0.05$.

not show any marked difference. The number of resorption sites was found significantly increased in all the groups compared with controls. Pre- and post-implantation loss was also significantly increased in all the groups compared to controls (Table 1).

3.1. Gross abnormality

There were significant gross structural abnormalities in group IV in the form of sub dermal hemorrhagic patches on the thoracic and abdominal areas, as well as kinky and short tails (Table 2).

3.2. Visceral abnormality

No gross visceral abnormality was seen in any of the treated groups.

3.3. Skeletal abnormality

Significant increases in the incidence of reduced ossification in parietal, interparietal and caudal bones were observed in the 750 ppm dosed group, whereas the 500 ppm dosed group revealed significant incidence of reduced ossification in caudal bones only (Table 2).

3.4. Chromium levels

Chromium levels were found to be significantly increased in the treated rats of group III and IV as evidenced by significantly higher metallic levels in maternal blood, placenta and fetuses (Table 3). The rate of transfer of chromium from the mother to placenta and from placenta to fetus, calculated as a ratio, revealed that in group IV the placental metal level was more than in the other treated groups which showed almost the same chromium transfer. The transfer ratio from placenta to fetus did not show any change in any of the treated groups.

3.5. Estrus cycle

The length of estrus cycle was increased due to chromium treatment in all the groups but was only significant in the highest dosed group (Table 4).

4. Discussion

In the present study, rats exposed to chromium through drinking water during the pregestational period revealed a reduced number of corpora lutea and implantations, retarded fetal develop-

Table 3
Chromium concentrations in different tissues of rats treated during the pregestational period

Tissue	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (μg/ml)	0.034 ± 0.007	0.049 ± 0.006^{a}	0.059 ± 0.008^{a}	0.192 ± 0.007^{abc}
Placenta (μg/g: fw)	0.093 ± 0.001	0.151 ± 0.008^{a}	0.168 ± 0.002^{ab}	0.232 ± 0.019^{abc}
Fetus (μg/g: fw)	0.042 ± 0.008	0.069 ± 0.007	0.163 ± 0.013^{ab}	0.241 ± 0.011^{abc}

Values represent mean $\pm$ S.E. of five rats in each group; fw, fresh weight.
Significance of the difference among various groups was evaluated by applying one-way ANOVA; Significance level: $p<0.05$; comparison between two groups: [a]vs. control; [b]vs. 250 ppm; [c]vs. 500 ppm.

ment and embryo- and fetotoxic effects as evidenced by the reduced number of fetuses (live and dead) per dam and higher incidence of still births, pre- and post-implantation loss in 500 and 750 ppm dosed mothers.

In our earlier study [8], a complete absence of implantation in 750 ppm treated mice was noted though reduced ovulation was present as evidenced by the significantly reduced number of corpora lutea. Mating was noticed in the 750 ppm group indicating that chromium treatment did not drive all the mice acyclic. The present study shows a species difference in the sensitivity between rats and mice. The chromium exposure (750 ppm) to mice showed a more significant effect on the duration of estrous cycle (72%) as compared to that in rats (37%). A differential effect on the implantation has also been observed in the two species but no correlation has been established yet.

The litter size in the 500 and 750 ppm dose groups was significantly reduced. This may be due to the effect of chromium (VI) on preimplantation embryos as evidenced by the study of Jacquet and Draye [20]. The maternal chromium is reported to pass freely through the placenta to the growing fetus as evidenced earlier from the analysis of bones from 120 human embryos in which the chromium content increased with age [7]. The levels of chromium used in the present study are not usually found in the environment but may be encountered at the work place or in effluents from the industrial establishments (40–50 000 ppm) [1].

Earlier studies have reported impaired gestational development when chromium was administered parenterally. Gale [21] injected 8 mg chromium trioxide per kg intravenously in hamsters on day 8 of gestation and found increased incidence of cleft palate.

In the present study, chromium accumulation in the fetuses of the 500 and 750 ppm groups might be attributed to the excessive transfer from maternal blood through placenta to fetus as evidenced by the placental/fetal chromium ratio. Therefore, the impaired fetal physiology in group III and IV resulting in embryo- and fetotoxic effects might be due to chromium accumulation as also seen with other heavy metals (Hg, Cd) and other xenobiotics [22]. Chromium (VI) is more readily transferred to the embryo and fetus [6,23] and is reported to produce teratogenic effects probably due to higher embryonic concentration [23].

The length of the estrus cycle was significantly increased in the highest dosed group (750 ppm). This might be correlated with the reduced number of ovulations observed in the highest dosed group as has already been reported and explained for chromium (VI) [24] and other chemicals [18]. The length of estrus cycle was also prolonged due to cadmium administration as reported by Baranski and Sitarek [25].

Danielsson et al. [23] studied the embryonic and fetal levels of chromium in early and late gestational stages of mouse and reported high placental chromium and increased passage to the fetus thereby affecting directly the embryonic structures. The lack of any marked teratological changes in the pre ent study compared to other investigators who exposed dams through parenteral administration, may be due to diminished uptake of chromium through the intestinal wall [26], when administered through drinking water. The absorption of some chromium through the

Table 4
Effect of chromium on the duration of estrus cycle

	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Estrus cycle (days)	5.2 ± 0.2	5.4 ± 0.7	5.7 ± 0.6	7.1 ± 0.5[a]

Value represents mean ± S.E. of 10 rats in each group.
Significance of the difference among various groups was evaluated by applying one-way ANOVA; significance level: $*p<0.05$; comparison between two groups: [a]vs. control.

intestine in experimental animals and humans is well documented [27] and chromium (VI) is absorbed to a greater extent than chromium (III) through the gastro-intestinal tract [28]. Coogan et al. [29] reported higher tissue levels of chromium (VI) compared to chromium (III) which reflects the greater tendency of chromium (VI) to traverse the plasma membrane and bind to the intracellular protein in various tissues, and this may explain the greater degree of toxicity associated with chromium (VI). Embryonic and fetal levels of chromium (VI) after chromate exposure to pregnant rats is reported to be 10 times greater [23] than that found after exposure to corresponding doses of chromium (III).

Therefore, the present study indicates that sufficiently high chromium (VI) intake through drinking water pregestationally affects the embryonic and fetal development in rats and mice differently, the latter being more sensitive. Pregestational exposure causes deleterious effects during the process of preimplantation embryonic development and thereby implantation, while exposure during later development increases the number of resorbed and dead fetuses.

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Table 2
Incidences of gross and skeletal abnormalities in the pups of chromium-treated rats during the pregestational period

Parameter	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Gross abnormalities				
Number of pups/litter observed	72/10	70/10	51/10	19/10
Drooping wrist	0	0	0	6/4 (32)
Sub-dermal hemorrhagic patches	0	0	8/6 (16)	8/4 (42)[a]
Kinky tail	0	0	0	8/6 (42) [a]
Short tail	0	0	4/4 (9)	10/4 (53)[a]
Skeletal abnormalities				
Number of pups/litter observed	48/10	45/10	34/10	19/10
Reduced parietal ossification	0	0	0	12/10 (63)[a]
Reduced inter-parietal ossification	0	0	0	10/10 (53)[a]
Reduced caudal ossification	6/4 (12)	8/5(18)	18/8 (53)[a]	18/10 (95)[a]

Gross and skeletal abnormalities are represented as number of abnormal pups/litter observed: percentage in parentheses calculated by the total number of pups observed.
Statistical significance evaluated by Fisher's Exact test; comparison between two groups. [a]vs. control. Significance level: $p < 0.05$.

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Table 2. Incidences of gross and skeletal abnormalities in the pups of dams treated with chromium during the pregestational period.

Parameters	Group I (Control)	Group II (250 ppm)	Group III (500 ppm)
Gross abnormalities			
Number of pups/litters observed	72/10	51/10	19/10
Drooping rist	0/10	0/10	6/4 (32)
Sub-dermal hemorrhagic patches	0	8/6 (16)	8/4 (42) a*
Kinky tail	0	0	8/6 (42) a*
Short tail	0	4/4 (9)	10/4 (53) a*
Skeletal abnormalities			
Number of pups/litter observed	48/10	34/10	19/10
Reduced parietal ossification	0	0	12/10 (63) a*
Reduced inter-parietal ossification	0	0	10/10 (53) a*
Reduced caudal ossification	6/4 (12)	18/8 (53) a*	18/10 (95) a*

Gross and skeletal abnormalites are represented as number of abnormal pups/litters observed.

The statistical significance was evaluated by Fisher's Exact test (Drunning and Kintz 1977).

Percentage in parentheses calculated by the total number of pups observed.

* Significance $p < 0.05$. Comparison between two groups: a-vs control.

Table 3
Chromium concentrations in different tissues of rats treated during the pregestational period

Tissue	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (μg/ml)	0.034 ± 0.007	0.049 ± 0.006[a]	0.059 ± 0.008[a]	0.192 ± 0.007[abc]
Placenta (μg/g: fw)	0.093 ± 0.001	0.151 ± 0.008[a]	0.168 ± 0.002[ab]	0.232 ± 0.019[abc]
Fetus (μg/g: fw)	0.042 ± 0.008	0.069 ± 0.007	0.163 ± 0.013[ab]	0.241 ± 0.011[abc]

Values represent mean ± S.E. of five rats in each group; fw, fresh weight.
Significance of the difference among various groups was evaluated by applying one-way ANOVA; Significance level: $p < 0.05$; comparison between two groups: [a]vs. control; [b]vs. 250 ppm; [c]vs. 500 ppm.

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Table 3. Chromium concentrations in different tissues of mice treated during the pregestational period

Tissue	GroupI (Control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (μg/mL)	0.03 ± 0.007	0.05 ± 0.006 a*	0.06 ± 0.008 a*	0.13 ± 0.007 abc*
Placenta (μg/g:f.w.)	0.09 ± 0.001	0.14 ± 0.008 a*	0.17 ± 0.002 ab*	No implantation
Fetus (μg/g:f.w.)	0.04 ± 0.008	0.07 ± 0.007	0.16 ± 0.013 ab*	No implantation

Values represent mean ± S.E of 5 mice in each group.

The significance of the difference among various groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz 1977). * Significance $p < 0.05$.

Comparison between two groups: a -vs control; b -vs 250 ppm; c -VS 500 ppm. f.w. q fresh weight.

UDC [615.31:547.264].015.6

P. A. Kolesnikov, Candidate of Medical Sciences

HABITUATION TO BUTYL ALCOHOL

Novosibirsk Research Branch of the Plastopolymer Association, Novosibirsk

The effect that butyl alcohol has on the body in animals and humans has not been sufficiently studied, especially with respect to long-term inhalation exposure in small concentrations. We conducted an experimental study on butanol habituation during long-term inhalation of its vapors in small concentrations. The experiments were performed on male white mice. The animals were allocated into 4 equal groups. The first was not subjected to butyl alcohol exposure and served as the control. The second group inhaled butanol vapors in a concentration of 0.78 ± 0.05 mg/m^3. The third group received a concentration of 6.6 ± 0.39 mg/m^3 and the fourth was exposed to a concentration of 40 ± 42 mg/m^3. Each group comprised 10 – 12 animals. In addition, 8 male rats were placed in each cage of the chambers in order to ascertain butanol's effect on the acid resistance of red cells.

Dynamic priming was done continuously in a chamber measuring 0.46 m^3. The control group of animals was placed in an identical chamber through which room air was drawn. The butanol concentration in the chambers was measured 4 times per week on the Tsvet-4 gas chromatograph. Habituation was judged according to change in the duration of hexenal-induced sleep and the toxicity of the butanol after 30 days of the experiment. The solution of hexenal was injected intraperitoneally using a computation of 60 mg/kg of the animal's body weight. Butyl alcohol was injected intragastrically based on the median lethal dose. It was preliminarily determined that its median lethal dose for mice is equal to 2.68 g/kg. The method of I. A. Terskov and I. I. Gitelzon was used to determine the acid resistance of the red cells.

The experiments demonstrated that the continuous inhalation of butanol vapors, even in a concentration at least 10 times smaller than the maximum allowable concentration for industrial facilities, was a relevant factor for the animals. Thus, after 30 days of butanol inhalation exposure, the duration of hexenal-induced sleep was decreased in comparison with the control, and the survival rate was increased with the intragastric administration of the substance's median lethal dose. At the same time, the extent of the changes in the parameters studied by us was different for each animal group. In the mice subjected to butanol inhalation exposure in a concentration of 0.78 mg/m^3, the difference from the control was insignificant, but the trend of the changes was exactly the same as that in the animals inhaling butanol vapors in higher concentrations. More pronounced changes were observed in mice inhaling butanol in concentrations of 6.6 mg/m^3 and 40 mg/m^3, even though the first concentration was less than the maximum allowable concentration by a factor of nearly 2, and the second one was only 4 times greater than the maximum allowable concentration. The toxicity of butanol during intragastric administration was significantly decreased ($P < 0.01$). The duration of hexenal-induced sleep declined in the first case by a factor greater than 2 ($P < 0.01$), while in the second case, this was almost by a factor of 2 ($P < 0.05$).

Butanol and hexenol are among those substances having a narcotic effect and, for this reason, it is valid for the changes we detected in the body's response to their one-time injection in large doses to be associated with narcotic habituation during long-term delivery in small doses. In the opinion of a number of researchers (I. D. Gadaksina et al.; Ye. I. Lyublina et al., and others), habituation is a relevant factor for animals and for humans. It is accompanied by stress of the body's compensatory responses, which are able to shift to decompensation at a certain stage of a toxin's effect. Some increase in the duration of hexenal-induced sleep in the animals exposed to butanol at the 40 mg/m^3 concentration, as compared to the animal group inhaling butanol vapors concentrated to 6.6 mg/m^3

evidently points to the appearance of a downward trend of the body's compensatory responses.

That butanol in a concentration of 0.78 mg/m^3 was relevant for the animals is also evidenced by a change in the acid resistance of the red cells in the rats. After 30 minutes, we found there to be a definite decrease in the percentage of stable red cells at the fourth minute ($P < 0.05$) in all animal subject groups along with some shifting of the erythrocyte histograms toward an increase of less stable red cells in rats inhaling butanol in the 6.6 mg/m^3 concentration, and there was an increase in the percentage of more stable red cells in the animals primed with butanol in a concentration of 0.78 mg/m^3. No erythrocyte histogram shift was detected in rats inhaling butanol in the highest concentration (400 mg/m^3).

Hence, with long-term inhalation exposure to a small concentration of butanol (6.6 mg/m^3) that is almost 2 times lower than the MAC for industrial facilities, there are definite changes in the duration of hexenal-induced sleep, the toxicity of butanol for mice, and the acid stability of rat red cells. Butanol in a concentration an order of magnitude lower than the maximum allowable concentration was also a relevant factor for the animals. Judging by the results of the experiment, there is a need for further research on the effect had on other systems of the body by long-term exposure to small concentrations of butanol.

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REDO
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Gigiena i sanitariia (Moscow, R.S.F.S.R. : 1943)
10479569

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ное водоснабжение села (в некоторых районах их использует от 88 до 97% сельского населения), свойственны процессы накопления нитратов как конечного продукта минерализации органических веществ. Вода 80,27% обследованных колодцев содержала нитраты в количестве, превышающем 10 мг N/л.

Установлена обратная зависимость между концентрацией нитратов в воде и глубиной колодцев (коэффициент корреляции 0,91). Исследование грунтовых вод по временам года позволило выявить прямую зависимость между содержанием нитратов в воде колодцев и количеством выпавших осадков (коэффициент корреляции 0,65). Выявленные закономерности свидетельствуют о том, что концентрация нитратов в воде обследованных колодцев отражает влияние степени загрязнения почвы сельских населенных пунктов.

Кроме того, изучены 27 383 истории новорожденных городских (Бобруйск, Минск) и 8 районных родовспомогательных учреждений Минской, Витебской и Могилевской областей, а также проведено сопоставление данных, характеризующих частоту врожденных пороков, диагностированных в перинатальном периоде в городской и сельской местности. В результате анализа полученных данных не выявлено существенных различий в удельном весе новорожденных с врожденными пороками развития плода среди сельских жителей по сравнению с городскими. Так, среди сельского населения процент врожденных пороков составил 1,67, среди городского — 1,10 ($P>0,05$). Исходя из того, что в статистических работах, проведенных в США и ГДР, содержатся данные о более высоком проценте врожденных пороков у городских жителей, а наш материал о количестве детей с этими пороками сравнительно мал, мы сопоставили вес детей жителей села и города при рождении. При сравнении количества доношенных новорожденных с относительно низким весом (до 3,5 кг) в городской (55,94%) и сельской (60,30%) местности не обнаружено существенных различий ($P>0,05$).

Следовательно, нет оснований рассматривать повышенное содержание азота нитратов в грунтовых водах как одну из причин, способствующих антенатальной гипотрофии плода.

В настоящее время в сельских населенных пунктах республики наметилась выраженная тенденция к переходу на централизованное водоснабжение за счет использования глубоких водоносных горизонтов, обладающих достаточной санитарной надежностью. Это послужит одной из радикальных профилактических мер, призванных оградить организм человека от вредного воздействия нитратов питьевой воды.

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Канд. мед. наук *П. А. Колесников*

ПРИВЫКАНИЕ К БУТИЛОВОМУ СПИРТУ

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Влияние бутилового спирта на организм животных и человека изучено недостаточно, особенно при длительном ингаляционном действии его в малых концентрациях. Нами проведено экспериментальное изучение привыкания к бутанолу при длительном вдыхании его паров в небольших концентрациях. Опыты проведены на белых мышах-самцах. Животные были распределены на 4 равные группы. 1-я группа не подвергалась действию бутилового спирта и служила контролем, 2-я вдыхала пары бутанола в концентрации 0,78± 0,05 мг/м³, 3-я — в концентрации 6,6± 0,39 мг/м³, 4-я — в концентрации 40± 42 мг/м³. Каждая группа состояла из 10—12 животных. Кроме того, в клетках камер размещали по 8 крыс-самцов для определения влияния бутанола на кислотную резистентность эритроцитов.

Затравку производили в камере емкостью 0,46 м³ динамическим способом, круглосуточно. Контрольную группу животных размещали в аналогичной камере, через которую протягивали комнатный воздух. Концентрацию бутанола в камерах определяли 4 раза в неделю на газовом хроматографе «Цвет-4». О привыкании судили по изменению длительности гексеналового сна и токсичности бутанола через 30 дней опыта. Раствор гексенала вводили внутрибрюшинно из расчета 60 мг/кг веса тела животного. Бутиловый спирт вводили внутрижелудочно из расчета среднесмертельной дозы. Предварительно было определено, что его среднесмертельная доза для мышей равна 2,68 г/кг. Кислотную резистентность эритроцитов определяли по методу И. А. Терскова и И. И. Гительзона.

Эксперименты показали, что круглосуточное вдыхание паров бутанола даже в концентрации, более чем в 10 раз меньшей предельно допустимой для производственных помещений, небезразлично для животных. Так, через 30 сут ингаляционного воздействия бутанола у всех подопытных животных по сравнению с контрольными уменьшилась длительность гексеналового сна и увеличилась выживаемость при внутрижелудочном введении среднесмертельной дозы вещества. При этом степень выраженности изменений исследованных нами показателей у каждой группы животных была различной. У мышей, подвергавшихся ингаляционному воздействию бутанола в концентрации 0,78 мг/м³, отличие от контроля было недостоверным, но направленность изменений была такой же, как и у животных, вдыхавших пары бутанола в более высоких концентра-

3 до 97% сельского населе-
продукта минерализации
одержала нитраты в коли-

нитратов в воде и глубиной
вых вод по временам года
тратов в воде колодцев и
Выявленные закономер-
де обследованных колод-
енных пунктов.
дских (Бобруйск, Минск)
ской и Могилевской обла-
щих частоту врожденных
ой и сельской местности.
енных различий в удель-
а среди сельских жителей
цент врожденных пороков
го, что в статистических
высоком проценте врож-
е детей с этими пороками
рода при рождении. При
низким весом (до 3,5 кг)
ено существенных разли-

держание азота нитратов
льной гипотрофии плода.
ки наметилась выражен-
счет использования глу-
арной надежностью. Это
ных оградить организм

Поступила 16/X 1974 г.

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У

«Пластполимер», Ново-

а изучено недостаточно,
нцентрациях. Нами про-
лительном вдыхании его
ышах-самцах. Животные
сь действию бутилового
трации 0,78±0,05 мг/м³,
2 мг/м³. Каждая группа
щали по 8 крыс-самцов
эритроцитов.
им способом, круглосу-
камере, через которую
их определяли 4 раза в
по изменению длитель-
. Раствор гексенала вво-
тиловый спирт вводили
льно было определено,
ую резистентность эрит-
.
паров бутанола даже
опустимой для произ-
через 30 сут ингаля-
по сравнению с кон-
ичилась выживаемость
При этом степень вы-
группы животных была
бутанола в концентра-
равленность изменений
ее высоких концентра-

циях. Более выраженные изменения наблюдались у мышей, вдыхавших бутанол в концентрациях 6,6 и 40 мг/м³, хотя первая концентрация почти в 2 раза меньше, а вторая — только в 4 раза больше предельно допустимой. Токсичность бутанола при внутрижелудочном введении значительно уменьшилась ($P<0,01$). Длительность гексеналового сна уменьшилась в первом случае более чем в 2 раза ($P<0,01$), а во втором — почти в 2 раза ($P<0,05$).

Бутанол и гексенол относятся к веществам наркотического действия, поэтому выявленные нами изменения в реакции организма на их однократное введение в больших дозах правомерно отнести к явлению привыкания к наркотику при его длительном поступлении в организм в небольших дозах. По мнению ряда исследователей (И. Д. Гадаскина и соавт.; Е. И. Люблина и соавт., и др.), привыкание небезразлично для животных и людей. Оно сопровождается напряжением компенсаторных реакций организма, которые на определенном этапе действия яда могут смениться декомпенсацией. Некоторое увеличение длительности гексеналового сна у животных, подвергавшихся действию бутанола в концентрации 40 мг/м³, по сравнению с группой животных, вдыхавших пары бутанола в концентрации 6,6 мг/м³, по-видимому, свидетельствует о появлении тенденции к уменьшению компенсаторных реакций организма.

О том, что бутанол в концентрации 0,78 мг/м³ небезразличен для животных, свидетельствует также изменение у крыс кислотной резистентности эритроцитов. Так, через 30 сут мы выявили достоверное уменьшение процента стойких эритроцитов на 4-й минуте ($P<0,05$) во всех группах подопытных животных с некоторым смещением эритрограмм в сторону увеличения менее стойких эритроцитов у крыс, вдыхавших бутанол в концентрации 6,6 мг/м³, и повышение процента более стойких эритроцитов у животных, подвергавшихся затравке бутанолом в концентрации 0,78 мг/м³. Смещения эритрограммы у крыс, вдыхавших бутанол в более высокой концентрации (400 мг/м³), не выявлено.

Таким образом, при длительном ингаляционном действии небольшой концентрации бутанола (6,6 мг/м³), которая почти в 2 раза ниже ПДК для производственных помещений, достоверно изменяются длительность гексеналового сна, токсичность бутанола для мышей и кислотная стойкость эритроцитов крыс. Бутанол в концентрации на порядок ниже предельно допустимой также небезразличен для животных. Судя по результатам опыта, необходимы дальнейшие исследования, касающиеся влияния длительного действия небольших концентраций бутанола на другие системы организма.

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Канд. мед. наук *Р. В. Петров*, *В. И. Воробец*

САНИТАРНО-ГИГИЕНИЧЕСКИЕ АСПЕКТЫ ПРИМЕНЕНИЯ НОВЫХ СРЕДСТВ БОРЬБЫ С ФИЛЬТРАЦИЕЙ НА ВОДОЕМАХ

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Исследованиями Украинского научно-исследовательского института гидротехники и мелиорации установлена возможность использования в качестве противофильтрационных добавок к грунтам кубовых остатков производства синтетических жирных кислот (КОСЖК) и спиртов (КОСЖС), являющихся отходами нефтеперерабатывающей промышленности и предприятий, выпускающих моющие средства. Для решения вопроса об опасности применения препаратов было изучено влияние кубовых остатков на условия водопользования.

С целью исследования действия препаратов на органолептические свойства воды и санитарный режим водоемов кубовые остатки вносили в грунты ложа модельных водоемов в соответствии с технологической схемой организации противофильтрационной защиты. В воде модельных водоемов определяли органолептические, а также общепринятые и специфические физико-химические показатели качества воды, выяснены закономерности развития и отмирания сапрофитной микрофлоры. Установлено, что экраны, созданные кубовыми остатками в дозах 5 т/га, не оказывают существенного влияния на качество воды модельных водоемов. Доза КОСЖК, превышающая 5 т/га, способствует появлению в воде постороннего запаха интенсивностью 2—4 балла и нефтепродуктов в концентрациях 0,3—0,6 мг/л.

Дозы КОСЖС 7,5 и 10 т/га вызывают в воде интенсивную стимуляцию процессов биохимического потребления кислорода и развития сапрофитной микрофлоры, нарушают процессы аэрации, что приводит к снижению в воде растворенного кислорода. Влияние на эти показатели качества воды экранов, созданных КОСЖС, прямо связано с их способностью выделять в нее анионноактивные вещества в концентрациях 0,1—1 мг/л.

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Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments

Emma L. McConnell, Abdul W. Basit and Sudaxshina Murdan

Abstract

To use rodent models effectively in in-vivo investigations on oral drug and vaccine delivery, the conditions in the gastrointestinal tract must be understood. Some fundamental information is currently unavailable or incomplete. We have investigated the pH, water content and lymphoid tissue distribution along the gastrointestinal tract, as well as the stomach volume, as these were critical to our investigations on pH-responsive drug delivery and colonic vaccination. The observed values were compared with those in man as an indication of the validity of the rodent model. The mouse stomach pH was 3.0 (fed) and 4.0 (fasted), and the corresponding values in the rat were 3.2 (fed) and 3.9 (fasted). The mean intestinal pH was lower than that in man (< pH 5.2 in the mouse; < pH 6.6 in the rat). This brings into question the use of rodents in investigations on enteric-coated drug carriers targeted to the large intestine/distal gut. The water content in the gastrointestinal tract in the fed and fasted mouse was 0.98 ± 0.4 and 0.81 ± 1.3 mL, respectively, and in the fed and fasted rat was 7.8 ± 1.5 and 3.2 ± 1.8 mL. When normalized for body weight, there was more water per kg body weight in the gastrointestinal tracts of the mouse and rat, than in man. The stomach capacity was found to be approximately 0.4 and 3.4 mL for mice and rats, respectively. The low fluid volume and stomach capacity have implications for the testing of solid dosage forms in these animal models. Substantial amounts of lymphoid tissue analagous to small intestinal Peyer's patches were measured in the rat and mouse colon, showing the feasibility of colonic vaccination, a route which might prove to have different applications to the more commonly studied oral vaccines. The existence of lymphoid tissue in the mouse and rat caecum has also been reported.

Introduction

Animal models are used extensively in the pre-clinical testing of drugs and vaccines. Rodents (mainly rats and mice) are often used due to their small size and low cost. Rats, having a relatively larger size and greater capacity for blood samples, are more useful for bioavailability studies, whereas mice are often used for vaccination studies. Despite the extensive use of these animals, certain features are either unknown or inadequately characterized, although a number of aspects of the mouse and rat gastrointestinal (GI) physiology have been reviewed by Kararli (1995). During our investigations into pH-responsive drug release at different locations in the gastrointestinal tract, and into colonic vaccination, we identified several key elements of gastrointestinal physiology that needed clarification to enable the use of rat and mouse models in the in-vivo studies. These were the pH and fluid content along the gastrointestinal tract, the stomach volume and the presence of lymphoid tissue in the colon of the animal models.

The pH in the gastrointestinal tract is a crucial factor, affecting the stability and solubility of drugs and their absorption through the mucosa; unsuitable pH may cause the precipitation of acidic or basic drugs from solution, or the degradation of labile compounds. In addition, enteric-coated drug delivery systems for modified or targeted drug release are increasingly being investigated, for example using polymers such as polymethacrylate- and cellulose-based enteric coatings, which dissolve only when the pH of the environment exceeds a threshold level. In such a situation, knowledge of the

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gut pH of the experimental animal is critical. Previous reports on the pH of the rat gastrointestinal tract are conflicting (Smith 1965; Ward & Coaes 1987), while reports of pH in the mouse small and large intestinal tract were not found.

The fluid content of the gastrointestinal tract is another critical factor in the dissolution of drug from a dosage form, and the dispersion of solid-dosage forms. Lomas & Graves (1999) and Schiller et al (2005) suggested that water in the gut lumen of man was not homogeneously distributed; this implied that a dosage form would be in contact with varying amounts of fluid or indeed none at all during its passage through the gastrointestinal tract. To enable better animal study design and extrapolation to man, or to better explain dosage form/drug behaviour in the rodent model, knowledge of the water content in these animals is important.

In this study, we have investigated the stomach volume, fairly crudely, to give a rough indication of the volumes that may be administered orally to the animal models. To our knowledge, there are no reports of mouse and rat stomach volume, although maximum volumes to be administered by the oral route have been suggested (Wolfensohn & Lloyd 1994). Gelatin capsule shells and mini-tablets have been administered to rats (Hu et al 1999; Wong et al 2006). Knowledge of the animal stomach volume would enable calculation of dosage form:stomach volume ratio, which would give an indication of the likely fate of the dosage forms, with respect to disintegration and drug dissolution and absorption.

In our laboratories, we are also investigating colonic vaccination as it may have different applications to the more commonly studied oral vaccines, which are expected to be processed mainly by the small intestinal immunological system. Like the small intestine, the colon contains gut-associated lymphoid tissue. In man, there are approximately 339 Peyer's patches (Cornes 1965) in the small intestine, and approximately 12 000–18 000 follicles in the large intestine (Langman & Rowland 1986, 1992; Gebbers et al 1992). Presence of such a large number of follicles in man's colon implies the feasibility of vaccine uptake and processing in the colon. Very little is known, however, about colonic vaccine uptake, although significant differences between the large and small intestinal immunological environments have been reported. For example, a predominance of IgA2 cells over IgA1 cells is seen in the colon (as in the rectum and in the female genital tract) in contrast to a predominance of IgA1 cells in the small intestine (McGhee et al 1999). Other benefits of colonic targeting include the decreased proteolytic activity which may be beneficial for sensitive antigens and the higher transit time, which could lead to prolonged antigen contact with the lymphoid tissue and thereby increased uptake. Before mice and rats can be used in studies on colonic vaccination, the presence and density of lymphoid tissue in the colon must be established. Although the lymphoid tissue in the small intestine of mice and rats has been well quantified (Hillery et al 1994; Florence et al 1995; Abe & Ito 1977), the lymphoid tissue in the large intestine has not, and has been reported here.

Materials and Methods

Animals

All procedures were approved by the School's Ethical Review Committee and were conducted in accordance with the Home Office standards under the Animals (Scientific Procedures) Act 1986.

Adult female Balb/c mice (18–22 g) and adult female Wistar rats (160–190 g) were purchased from Harlan Olac Ltd. The animals were fed on Teklad Global 18% Protein Rodent Diet, from Harlan Olac Ltd.

Preparation and dissection procedure

Groups of animals (n = 5–8) were fasted overnight with free access to water, while other groups were allowed access to food and water at all times. The mice were killed by a Schedule One method (CO_2 asphyxiation), after which the intestinal tract was immediately removed and divided into sections: the stomach, the small intestine (into three sections approximating to the duodenum, jejunum and ileum), the caecum and the colon (into two sections approximating to the proximal and distal colon). Subsequently, the pH, water content and lymphoid tissue density of the different sections was measured as follows.

Determination of pH of gastrointestinal contents

The contents of each gastrointestinal section were removed, mixed and the pH was determined using a pre-calibrated pH 211 Microprocessor pH Meter (Hanna Instruments). pH measurements were taken a total of three times with the gastrointestinal tract contents being re-mixed, the pH meter being washed with distilled water and the calibration checked between measurements. An HI 1333 probe was used, with a spherical tip (diameter 7.5 mm); it was ensured that the sample covered the probe tip, and a stable reading acquired. The order in which the pH of the different gastrointestinal tract sections was read was varied within each group to minimize any influence of post-mortem time on pH.

Determination of pH of standard rat/mouse chow

To determine the influence of the animal feed on the pH of the gastrointestinal contents, the pH of standard rat/mouse chow was measured. Three pieces of standard mouse/rat chow (9.17 g) were mixed with 10 mL of tap water until the food pellet had disintegrated, and the pH of the resulting mixture was measured using the same pH meter.

Determination of water and solid contents of the gastrointestinal tract

To determine the gastrointestinal water and solid contents, the wet mass of the section contents was recorded, followed by lyophilization (Virtis-Advantage Freeze Drying Apparatus, Virtis, UK), measurement of the dry mass and calculation of water content.

Determination of stomach capacity

Approximate values for the volume of the mouse and rat stomach were determined by filling the stomach with distilled

water, and observing the results. The aim was to produce a rough estimate, as the method could only give a crude assessment of the volume, and was subject to investigator bias. The stomach, hand-held shut at the pyloric opening, was filled, using a syringe, via the oesophagus until it was considered to be comfortably full, with no obvious stress on the tissue (1), stretched (2), or to the point of bursting (or could no longer be filled) (3).

Determination of lymphoid tissue patches along the gastrointestinal tract

The method of Langman & Rowland (1986) was used. The emptied gastrointestinal sections were placed into glass vials containing 20 mL 10% v/v aqueous acetic acid and incubated overnight in the refrigerator. Acetic acid was used as it enhanced the visualization of the lymphoid tissue. The following day, the gastrointestinal tract sections were removed, opened lengthways, blotted dry and photographed, and the numbers of individual lymphoid follicles and patches (collections of follicles) were counted. The mean number of patches or follicles per cm was calculated from the data for the individual animals.

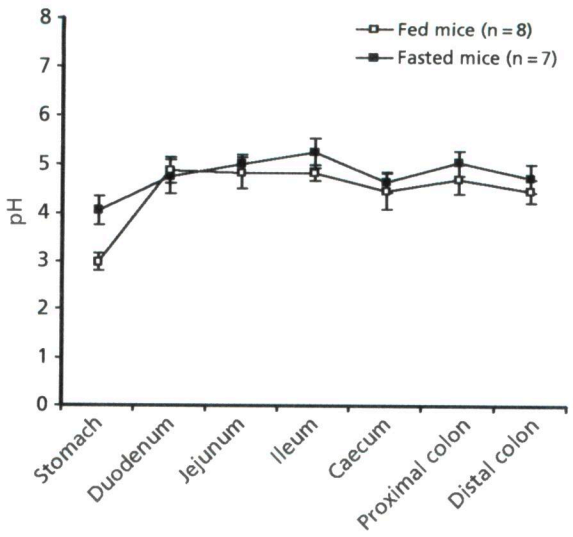

Figure 1 pH values along the mouse gastrointestinal tract. Mean and error bars are shown.

Statistical analysis

The data gathered from mice was analysed using parametric tests. The influence of fed (n = 8) and fasted (n = 7) states on mouse gastrointestinal pH, and water and solid contents were analysed using Student's Independent *t*-test. Differences between gastrointestinal tract sections for pH and water content were analysed using one-way analysis of variance, with post-hoc analysis using Tukey's test.

The data obtained from rats was analysed using non-parametric tests, as the data did not fulfil the assumptions required for parametric tests. The influence of fed (n = 5) and fasted (n = 5) state on rat gastrointestinal pH, and water and solid contents were analysed using the Mann–Whitney *U*-test. The differences between gastrointestinal sections for pH and water content were analysed using Kruskal–Wallis, with Nemenyi's post-hoc analysis.

All tests, apart from Nemenyi's test were carried out using SPSS Version 14.0 statistical software package. Nemenyi's test was conducted as described in Jones (2002). Results were considered statistically significant when $P < 0.05$.

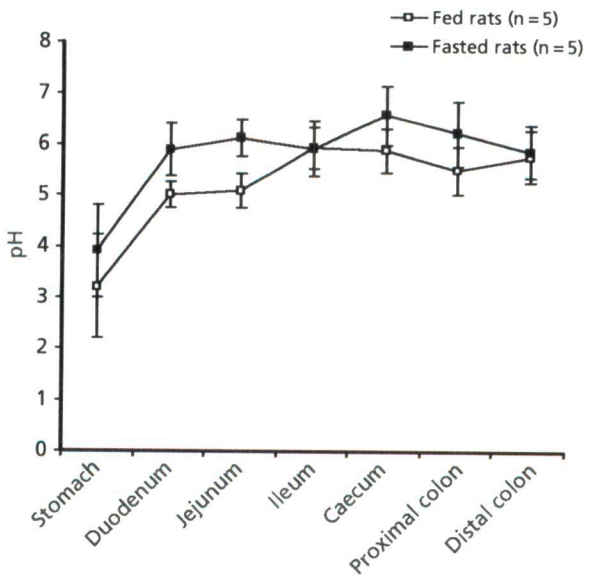

Figure 2 pH values along the rat gastrointestinal tract. Mean and error bars are shown.

Results and Discussion

The pH along the gastrointestinal tract of mice and rats

The pH of the contents of the different gastrointestinal sections of fed and fasted mice and rats are shown in Figures 1 and 2, and in Table 1. The standard deviations showed variability between individuals. Such variability has been observed in man (Evans et al 1988; Fallingborg et al 1989). The lowest pH was seen in the stomach, in both rats and mice. In both animals, the stomach pH appeared higher in the fasted state (3.9 compared with 3.2 in rats and 4.0 compared with 3.0 in mice), although the difference was only statistically significant in the mouse. Higher pH in the fasted state was surprising given that, in man, the fasted gastric pH is lower than the fed gastric pH (fasted pH 1.7 increasing to 5.0 after meal ingestion in healthy subjects (Dressman et al 1990; Russell et al 1993)) due to the buffering effects of food (Malagelada et al 1976). However, this was dependent on the meal type, with high protein meals having increased buffering effect over an isocalorific carbohydrate meal (Richardson et al 1976). In this study, the mice and rats were fed on a standard low protein (18%), low fat (5%) diet. The low

Table 1 The pH values of the mouse and rat gastrointestinal tract

Gastrointestinal section	pH mean (s.d.)			
	Mice		Rats	
	Fed	Fasted	Fed	Fasted
Stomach	2.98 (0.3)	4.04 (0.2)	3.20 (1.0)	3.90 (1.0)
Duodenum	4.87 (0.3)	4.74 (0.3)	5.00 (0.3)	5.89 (0.3)
Jejunum	4.82 (0.2)	5.01 (0.3)	5.10 (0.3)	6.13 (0.3)
Ileum	4.81 (0.3)	5.24 (0.2)	5.94 (0.4)	5.93 (0.4)
Caecum	4.44 (0.2)	4.63 (0.4)	5.90 (0.4)	6.58 (0.4)
Proximal colon	4.69 (0.3)	5.02 (0.3)	5.51 (0.5)	6.23 (0.4)
Distal colon	4.44 (0.3)	4.72 (0.2)	5.77 (0.5)	5.88 (0.5)

protein content of the animals' diet could be responsible for the absence of a food buffering effect. The pH of rat chow in water was 5.86 ± 0.06 and was therefore not responsible for the lower pH observed in the fed state. In addition, while the reasons for the difference between man and rodents are not clear, it is obvious that during experiments in man, fed and fasted states can be controlled more closely. In contrast, although the fed-state mice have free access to food, it is not known at what time they last ingested food, and in what quantity, and the immediate buffering effects of food may not have been observed.

The pH of the small intestinal contents also appeared to be higher in the fasted state than in the fed state, but this was not statistically significant in rats or mice. This suggested that the fed state of the animal had no effect on intestinal pH, which is similar to the situation in man, where the small intestinal and colonic pH are variable, but differences are not largely associated with the fed or fasted states (Kalantzi et al 2006). As expected, the small intestinal pH was higher than the gastric pH, due to the secretion of pancreatic juice and buffering with bicarbonate ions. In mice, there was a small drop in pH in the caecum. This may be associated with the increased presence of short chain fatty acids produced by bacterial polysaccharidases, bacteria being present in greater numbers in the caecum. Such a pH drop in the caecum also occurs in man (Evans et al 1988). Overall, the mean intestinal pH of both mice and rats does not reach the pH values reported in man i.e. 7.5, 6.4 and 7 in the distal small intestine, caecum and colon, respectively (Evans et al 1988).

The stomach pH values for the rat and mice were similar to that reported by Smith (1965). In contrast, the mean intestinal pH values of both animals were lower than expected and did not reach the values of pH 6–8 that have been reported in the literature for rats (Smith 1965; Ward & Coates 1987), though some individual rat pH values were found to be above pH 7. Differences in the methodology may help explain the different values obtained. Smith (1965) mixed distilled water with rat gut contents, while Ward & Coates (1987) inserted a pH probe into sections of excised rat gastrointestinal tract. In our investigation, mixing of undiluted contents was carried out, which may be more representative of the pH that a drug or delivery system is exposed to, due to the continually moving intestinal contents. To our knowledge, this is the first report of the pH of mouse intestinal tract contents.

The low intestinal pH in mouse and rat has implications for the in-vivo testing of oral pharmaceuticals in these animals. For example, drugs which require a basic pH to dissolve may precipitate at the lower pH values seen in the mouse or rat. This may prevent drug absorption and pharmacokinetic extrapolation to man would be inaccurate. The lower pH seen in mice and rat gastrointestinal tract also has implications when pH-responsive drug carriers are being investigated. For example, the pH responsive polymethacrylate polymers such as Eudragit S and FS, which dissolve at pH 7.0, but are water-insoluble at lower pH, are being investigated to target drug release to the distal intestinal tract e.g. for the treatment of diseases such as ulcerative colitis (Basit 2005; Ibekwe et al 2006). The low pH values for the mouse and rat gastrointestinal tract shown in this paper ($pH < 7.0$) suggest that rats and mice may not be the most appropriate models for the study of pH sensitive dosage forms targeted to the human lower intestine and colon, where pH is often greater than 7.0.

The water and solid contents of the mouse and rat gastrointestinal tract

The contents of the gastrointestinal tract are generally semi-solid. Water, either ingested or secreted, exists as fluid in the gastrointestinal tract. In this study, we measured the water content by freeze drying; the solid and water contents of the gastrointestinal tract of mice and rats are shown in Figures 3 and 4, respectively. As expected, total contents of the gastrointestinal tract were greater in rats than in the smaller mice. There were more solid contents in the fed rat gastrointestinal tract than in the fasted rat. Water content was also higher in the fed state, possibly due to increased secretions and water bound with the ingested food. The total amount of water present in the rat gastrointestinal contents was similar to that reported by Cizek et al (1954), who measured water by evaporating gastrointestinal contents to dryness and reported that gut water represented 1.8% (fasted) and 4.5% (fed) of total body weight (198–232 g) of female rats. In the mouse, differences between the total solid and water contents were less

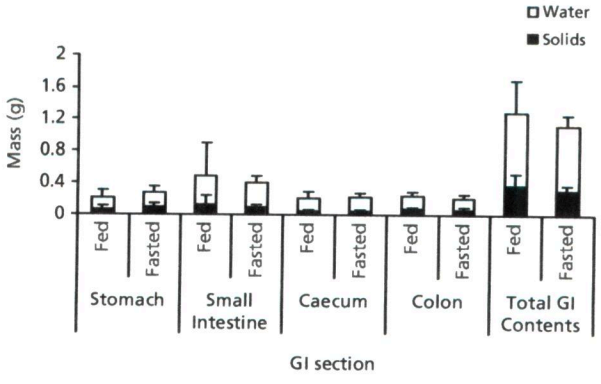

Figure 3 Water and solid compositions of the mouse gastrointestinal tract contents. Mean and error bars are shown.

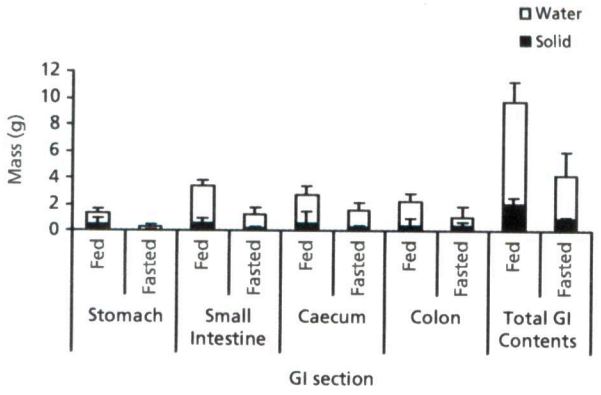

Figure 4 Water and solid content of the rat gastrointestinal tract contents. Mean and errors bars are shown.

obvious between the fed and fasted states, the small quantities making it more difficult to ascertain differences. As expected, the water concentration decreased along the length of the gastrointestinal tract from 82% w/w of the small intestinal contents to 71% w/w in the colon in rats, and from 74 to 67% w/w along the same segments in mice. The decreasing water content was observed visually as an increase in the viscosity of the gastrointestinal contents.

In Figures 3 and 4, the most striking observation was the very low levels of fluid present along the gastrointestinal tract, the total mass of water in the mouse gut being less than 1 mL (0.98 ± 0.4 mL fed, 0.81 ± 1.3 mL fasted). In experiments where mice are orally dosed with solid or semi-solid drug delivery systems, the latter may not come into contact with enough fluid to disperse and/or dissolve. The rat seems a more appropriate model for the dissolution of drug delivery systems, which require contact with sufficient water. The larger water content in the fed rat (7.8 ± 1.5 mL), compared with the fasted rat (3.2 ± 1.8 mL) suggests that if a dosage form is being investigated in the rat model, it may be beneficial to deliver it in the fed state, although interactions of food with drug or with dosage form may mean that this is not appropriate in all circumstances.

To compare with human data, the mass of rodent intestinal contents with respect to total body mass has been calculated. In man, the total large intestinal (colonic and caecal) water content post-mortem was found to average 187 g, or 2.6 g kg^{-1} body mass assuming a 70-kg body weight. For an average rat (175 g), the average (fed and fasted) colonic water content was 7.14 g kg^{-1} or 16.9 g kg^{-1} when the caecum was included. For an average (fed and fasted) mouse, the values were 7.8 g water kg^{-1} body weight and 16.3 g kg^{-1} when the caecal contents were counted. In man, the small intestine has been reported to contain a total of 206 g water or 3.8 g kg^{-1} (Gotch et al 1957). This compared with 11.1 g water kg^{-1} body weight in the rat small intestine, and 16.5 g water kg^{-1} body weight in the mouse small intestine. The same authors found 118 g water in the stomach or 2.2 g water kg^{-1} body weight. In our study, the corresponding values were 3.2 g kg^{-1} in rats and 8.5 g water kg^{-1} body weight in mice. Thus, when the values were normalized to take into account total body mass, more water per kg body weight was found in the gastrointestinal tracts of the mouse and the rat than in man.

Interestingly, although the total water content reported in the small and large intestine in man was high (206 g (Gotch 1957) and 187 g, respectively (Cummings et al 1990)), Schiller et al (2005), using magnetic reasonance imaging, measured a median free fluid volume of 105 ± 72 mL (fasted) and 54 ± 41 mL (fed) in the small intestine, and 13 ± 12 mL (fasted) and 11 ± 26 mL (fed) in the colon. These values indicated that most of the gut water was in the bound state. This suggested that only a proportion of the water content was available for drug or dosage form dissolution, and the same is likely to be true of the water content in the animal models discussed.

The volume of the mouse and rat stomach

Drug or vaccine formulations are often given to experimental animals by oral gavage. Consequently, the volume of the stomach is considered an important parameter for oral dosing, and the results are shown in Table 2. The mouse stomach was approximately one-tenth the volume of the rat stomach. Wolfensohn & Lloyd (1994) have suggested the upper limit for oral dosing in mice to be 20 mL kg^{-1}. Thus, for a mouse of 20 g, the maximum oral dosage volume would be 0.4 mL. For rats, the recommended maximum is 10 mL kg^{-1}; for a 200 g rat this would give a dosing volume of 2 mL. These values correlate to some degree with the 'comfortably full' volumes shown in Table 2, despite the fact that post-mortem results would be likely to differ from an in-vivo situation, since elasticity and responsiveness of gastric tissue to pressure may be altered.

Quantification of lymphoid tissue along the gastrointestinal tract of the mouse and rat

The lymphoid tissue along the gastrointestinal tract can be categorized broadly into, firstly, individual lymphoid follicles, which are seen as raised white areas, and secondly into patches, which are collections of individual follicles. In the small intestine these are referred to as Peyer's patches. No lymphoid tissue was observed in the stomach. However, significant amounts of lymphoid tissue were observed in the mouse and rat caecum (Table 3 ; Figure 5A, B). Thus, we confirmed previous reports on the presence of lymphoid tissue in mouse caecum (Owen et al 1991) and have reported, for the first time to our knowledge, the presence of lymphoid tissue in rat caecum.

Table 2 Fill volumes of mouse and rat stomach

	Volume (mL (s.d.))	
	Mice (n = 10)	Rats (n = 8)
1. Comfortably full	0.37 (0.09)	3.38 (0.52)
2. Stretched	0.55 (0.09)	4.63 (0.44)
3. On the point of bursting/could not be expanded further	0.71 (0.11)	6.63 (0.92)

Table 3 Quantification of lymphoid tissue in the intestinal tract of Balb/c mice and Wistar rats. The mean and (range) values are shown

	Mouse (n = 15)			**Rat (n = 10)**		
	Small intestine	Caecum	Colon	Small Intestine	Caecum	Colon
Mean length (range)	34.5 (29–39)	–	11.5 (9–14)	82.8 (70–97)	–	13.9 (12–18)
Mean number of patches (range)	10.1 (3–15)	1.4 (1–5)	11.6 (7–15)	9.4 (7–15)	1.2 (1–2)	3.8 (2–11)
Mean number patches cm^{-1}	0.3	–	0.8	0.33	–	0.3
Mean number of follicles (range)	57.5 (22–80)	18.1 (9–26)	39.4 (18–54)	207.5 (142–273)	13.5 (8–26)	38.6 (16–83)
Mean number follicles per patch	5.7	12.9	3.4	30.6	12	28.5
Mean number follicles cm^{-1}	1.6	–	3.4	2.1	–	3.4

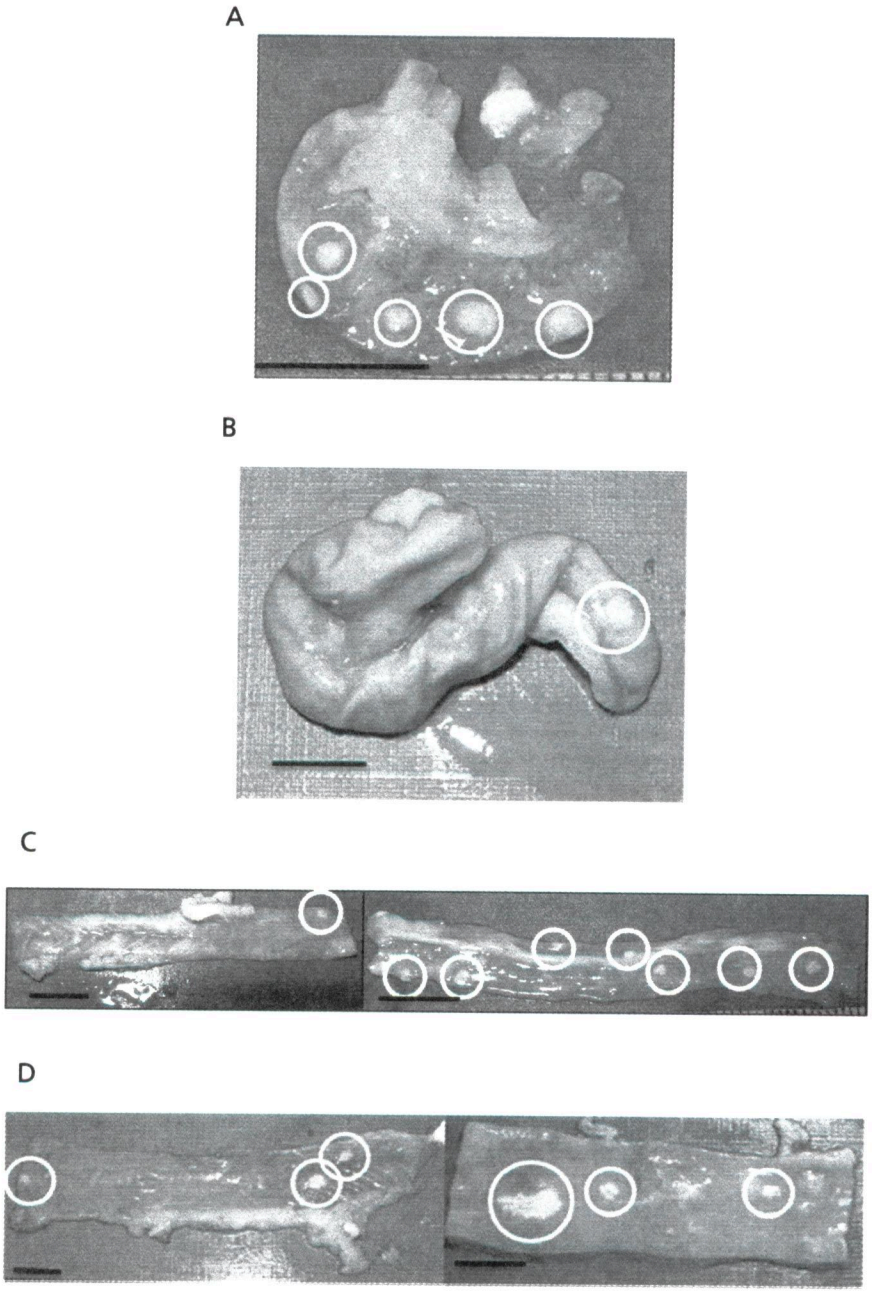

Figure 5 Lymphoid tissue patches in the (A) caecum of a Balb/c mouse (scale bar = 10 mm), (B) caecum of a Wistar rat (scale bar = 10 mm), (C) colon of the Balb/c mouse (left proximal; right distal) (scale bar = 10 mm), (D) colon of the Wistar rat (left proximal; right distal) (scale bar = 10 mm).

The numbers of Peyer's patches in the mouse and rat small intestine (Table 3) were similar to those reported in the literature: 6–12 Peyer's patches in mouse small intestine (Abe & Ito 1977) and 15 Peyer's patches in the rat small intestine (Hillery et al 1994; Florence et al 1995). The values for the number of lymphoid patches in the mouse colon, however, are slightly less than a previously reported value of 1.4 patches cm^{-1} (Owen et al 1991).

Examination of lymphoid tissue density along the gastrointestinal tract revealed that in rats and mice, Peyer's patches were distributed randomly along the sections of the small intestine, with no predilection for a particular area ($P > 0.05$). Examination of the three small intestinal sections (roughly duodenum, jejunum and ileum) within each animal showed that there were similar numbers of patches and follicles per cm, in all three sections (data not shown). Similarly, there was no difference between the number of patches per cm in the proximal and distal colon ($P > 0.05$), which correlated with the random distribution reported in man (Langman & Rowland 1986). Photographs illustrating the random distribution of patches in the mouse and rat colon are shown in Figure 5C, D.

There were, however, differences between the quantity of lymphoid tissue in the small intestine and colon. In mice, the number of patches in the small intestine and colon were similar, but the number of individual follicles was much greater in the small intestine. However, taking into account the lengths of the respective sections, there were actually more follicles and patches per cm in the colon ($P < 0.05$). In rats, there were significantly more patches and follicles in the small intestine, relative to the colon. Taking into account the large differences in intestinal tract length in the rat, similar numbers of patches per cm were seen between small intestine and colon ($P < 0.05$), and more follicles were found per cm in the colon ($P < 0.05$). Mouse colonic lymphoid patches tended to be smaller, containing fewer follicles than small intestinal ones. In contrast, rat lymphoid patches were of similar size in both the small and large intestine. The rat lymphoid patches were, in general, larger than mouse patches and contained a greater number of follicles. The presence of lymphoid tissue in the colon of mice and rats confirms that these animals could be used in colonic vaccination studies.

Conclusion

pH values of the small and large intestinal contents in mice and rats were lower than previously reported, and were lower than the pH levels in man. This has implications for the use of rats and mice in testing of drug formulations, such as pH-responsive drug carriers. The very low levels of fluid present in the mouse gastrointestinal tract cautions against the use of mice when drug dissolution from an oral dosage form is examined. The higher water levels in the rat, especially in the fed state, shows that the rat would be a more suitable animal model. Colonic lymphoid tissue was quantified and compared with small intestinal tissue, in both rats and mice. The significant quantity of lymphoid tissue in the colon in both animals highlights the colon as an immunologically important organ and shows that colonic vaccination may be studied in these animal models. Finally, the presence of lymphoid tissue in the mouse caecum was confirmed and its presence in rat caecum has been reported.

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Bohn, Brent

From: Kloc, Kenneth@OEHHA <Kenneth.Kloc@oehha.ca.gov>
Sent: Thursday, July 30, 2015 3:33 PM
To: Sasso, Alan
Subject: n-Butanol report reference

Hello Dr. Sasso,

I'm a toxicologist at Cal/EPA OEHHA currently reviewing information on n-Butanol for possible development of a non-cancer inhalation health screening value. I'm contacting you since you are listed as an author of USEPA's Draft Toxicological Evaluation of n-Butanol (2011). The draft reviews two articles translated articles that were originally published in Russian. I was wondering if you wouldn't mind providing OEHHA with a copy of these translations? The two references are:

1. Baikov, BK; Khachaturyan, MK. (1973) Hygienic evaluation of the reflex action on the body of low concentrations of butyl alcohol entering the atmosphere. Gig Sanit 38(12):7–11. (Russian)

2. Rumyantsev, AP; Ostroumova, NA; Astapoval, SA; et al. (1976) Sanitary toxicological features of butyl alcohol under conditions of prolonged inhalation route entry. Gig Sanit 11:12–15. (Russian)

Or please feel free to let me know if you cannot fulfill this request.

Best Regards,
Ken Kloc, Ph.D.

Bohn, Brent

From: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Sent: Wednesday, April 16, 2014 6:38 PM
To: Sasso, Alan
Cc: Wong, Patty@OEHHA; Gibbons, Catherine
Subject: PBPK Contact

Hi, Alan.
Thanks for providing us with your SOT poster – very interesting! We appreciate that you are willing to share your work with us and we look forward to discussing this further. Our PBPK person is Patty Wong. I've spoken with her and given her a heads up that you will be contacting her. She is on vacation this week and will not be returning to work until Monday, the 21st. You can reach her at:
patty.wong@oehha.ca.gov or (916) 323-2627. Thanks!
Elaine

Bohn, Brent

From: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Sent: Tuesday, November 25, 2014 1:49 PM
To: Sasso, Alan
Cc: Gibbons, Catherine
Subject: PBPK manuscript

Hi, Alan.
The topic of the manuscript you're currently preparing came up in one of my meetings yesterday and some folks here had some questions that I couldn't quite answer. I was wondering if you might have some time tomorrow (if you're even working) to have a short meeting to discuss the paper? If so, how does 1:00 pm your time sound? Please let me know and I'll set it up. Thanks!
Elaine

Elaine M. Khan, Ph.D.
Chief, Water Toxicology Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
MS-12B
P.O. Box 4010
1001 I Street
Sacramento, CA 95812
Tel: (916) 324-1277
Fax: (916) 327-7320
Email: elaine.khan@oehha.ca.gov

Bohn, Brent

From: Sasso, Alan
Sent: Monday, July 22, 2013 10:39 AM
To: Elaine.Khan@oehha.ca.gov
Cc: Gibbons, Catherine
Subject: PBPK model errata for hexavalent chromium

Hi Elaine,

The errata for the hexavalent chromium PBPK model is published in the most recent issue of Chemico-Biological Interactions (see below). The modeling changes were necessary after we reviewed the original manuscript and sent comments to the corresponding authors.

Let us know if you have any additional questions regarding our analysis of the PBPK modeling and data.

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
(703)-347-0179

From: Flowers, Lynn
Sent: Monday, July 22, 2013 8:12 AM
To: Gibbons, Catherine; Sasso, Alan; Cogliano, Vincent; Berner, Ted
Subject: Fw: Chemico-Biological Interactions: Alert 16 July-22 July

Note chromium correction at end in case you didn't already see it....

From: ScienceDirect Message Center <**valert@prod.sciencedirect.com**>
Sent: Monday, July 22, 2013 4:59:56 AM
To: Flowers, Lynn
Subject: Chemico-Biological Interactions: Alert 16 July-22 July


New articles in Chemico-Biological Interactions available on ScienceDirect

Chemico-Biological Interactions

New Articles in Press, 16 July-22 July 2013

Modify or Remove My Alerts

1. **Fenofibrate suppresses melanogenesis in B16-F10 melanoma cells via activation of the p38 mitogen-activated protein kinase pathway** Original Research Article

Available online 18 July 2013
Yu-Chun Huang, Kao-Chih Liu, Yi-Ling Chiou, Chao-Hsun Yang, Tien-Hui Chen, Ting-Ting Li, Li-Ling Liu

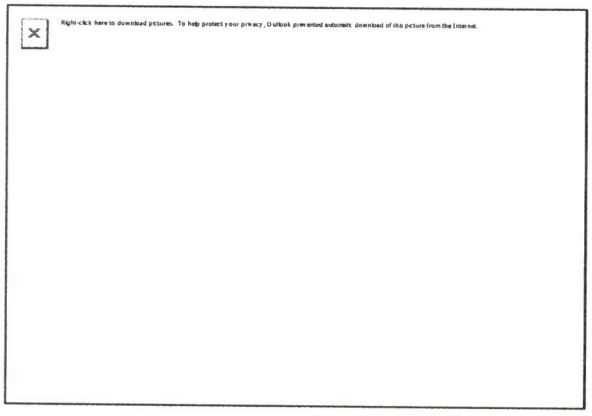
2. **Evaluation of the Anti-Inflammatory Activity of Riparin II (O-Methil-N-2-Hidroxi-Benzoyl Tyramine) in Animal Models** Original Research Article

Available online 17 July 2013
Alyne Mara Rodrigues de Carvalho, Nayrton Flávio Moura Rocha, Leonardo Freire Vasconcelos, Emiliano Ricardo Vasconcelos Rios, Marília Leite Dias, Maria Izabel Gomes Silva, Marta Maria de França Fonteles, José Maria Barbosa Filho, Stanley Juan Chavez Gutierrez, Francisca Cléa Florenço de Sousa

3. **Antioxidant, Metal-Binding and DNA-damaging Properties of Flavonolignans: A Joint Experimental and Computational Highlight based on 7-*O*-Galloylsilybin** Original Research Article

Available online 17 July 2013
Jan Vacek, Martina Zatloukalová, Thomas Desmier, Veronika Nezhodová, Jan Hrbáč, Martin Kubala, Vladimír Křen, Jitka Ulrichová, Patrick Trouillas

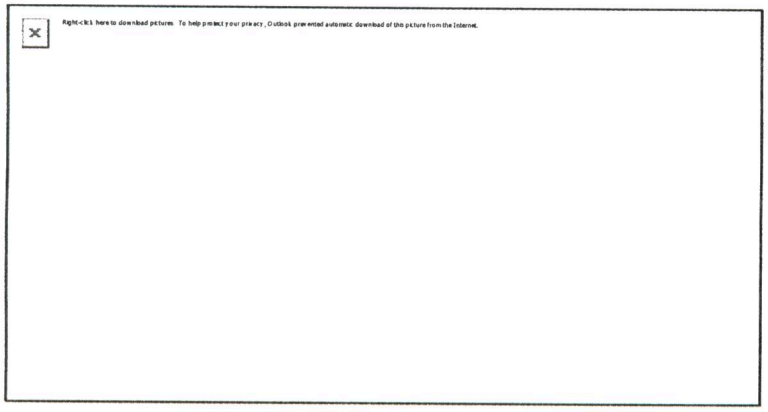
Graphical abstract



4. **Formononetin potentiates epirubicin-induced apoptosis via ROS production in HeLa cells in vitro** Original Research Article

Available online 16 July 2013
Yu-Li Lo, Wanjen Wang

Graphical abstract



5. **Synergistic effect of the L-tryptophan and kynurenic acid with dipyrone or paracetamol in mice** Original Research Article

   Available online 16 July 2013
   Nayrton Flávio Moura Rocha, Emiliano Ricardo Vasconcelos Rios, Alyne Mara Rodrigues Carvalho, Leonardo Vasconcelos Freire, Marília Leite Dias, Marta Maria de França Fontelesb, Francisca Cléa Florenço de Sousa

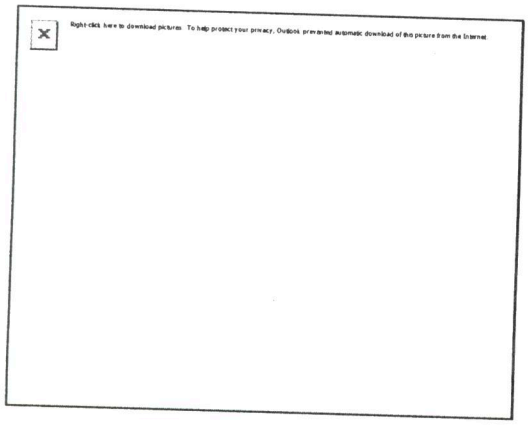
6. **The antioxidant effect of the mesoionic compound SYD-1 in mitochondria** Original Research Article

   Available online 16 July 2013
   Gustavo Jabor Gozzi, Amanda do Rocio Andrade Pires, Glaucia Regina Martinez, Maria Eliane Merlin Rocha, Guilhermina Rodrigues Noleto, Aurea Echevarria, André Vinicius Canuto, Sílvia Maria Suter Correia Cadena

7. **Platycodin D inhibits migration, invasion, and growth of MDA-MB-231 human breast cancer cells via suppression of EGFR-mediated Akt and MAPK pathways** Original Research Article

   Available online 16 July 2013
   Jaemoo Chun, Yeong Shik Kim

Graphical abstract



Chemico-Biological Interactions

Volume 204, Issue 3, Pages 135-206, 25 August 2013

1. **Inside front cover Editorial board**

   Pages IFC

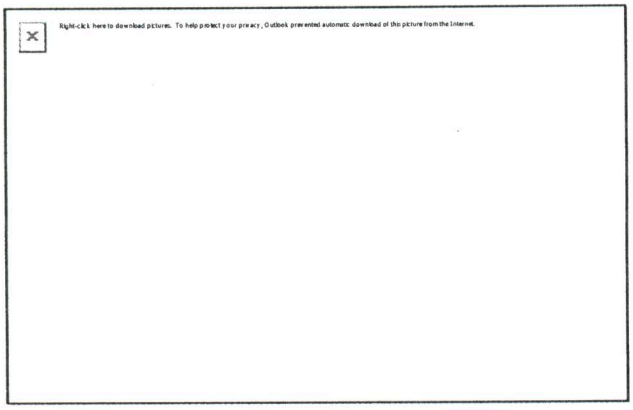
2. **Cytotoxic interaction between amiodarone and desethylamiodarone in human peripheral lung epithelial cells** Original Research Article

   Pages 135-139
   Fiona C. Roth, Jeanne E. Mulder, James F. Brien, Takashi Takahashi, Thomas E. Massey

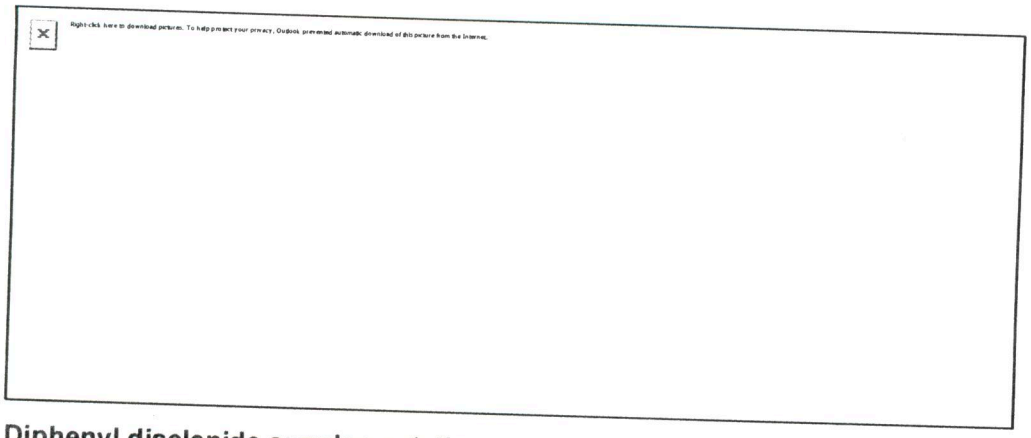
3. **In vitro metabolism of brucine by human liver microsomes and its interactions with CYP substrates** Original Research Article

Graphical abstract

The comparative study on the geometrical and electrostatic properties of the HAT inhibitors curcumin and cinnamoyl compounds in gas phase and amino acid environments give an insight on the molecular flexibility and the exact modification of electrostatic interaction of these inhibitors in the active site of p300. These fine details at the electronic level allow to understand the exact drug–receptor interactions.



Graphical abstract



9. **Diphenyl diselenide supplementation reduces biochemical alterations associated with oxidative stress in rats fed with fructose and hydrochlorothiazide** Original Research Article

   Pages 191-199
   Marinei Cristina Pereira Ribeiro, Daiana Silva Ávila, Viviane Patrícia Pires Schiar, Danúbia Bonfanti dos Santos, Daiane F. Meinerz, Marta Medeiros Frescura Duarte, Roger Monteiro, Robson Puntel, Andreza Fabro de Bem, Waseem Hassan, Nilda Berenice de Vargas Barbosa, João Batista Teixeira Rocha

10. **Disulfiram and its emerging role as an adjunctive anti-neoplastic agent**

    Pages 200
    Shailendra Kapoor

11. **Corrigendum to "Physiologically based pharmacokinetic model for rats and mice orally exposed to chromium" [Chem Biol Interact 2012;200:45–64]**

    Pages 201-206
    C.R. Kirman, S.M. Hays, L.L. Aylward, M. Suh, M.A. Harris, C.M. Thompson, L.C. Haws, D.M. Proctor

News Release

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH

FOR IMMEDIATE RELEASE
April 15, 2014
PH14-038

CONTACT: Anita Gore
Heather Bourbeau
(916) 440-7259

CDPH Submits Final Regulation Package Regarding Hexavalent Chromium (Cr VI) and Drinking Water

SACRAMENTO - The California Department of Public Health (CDPH) today submitted to the Office of Administrative Law (OAL) its final proposed regulation establishing the first ever drinking water Maximum Contaminant Level (MCL) for hexavalent chromium (Cr VI). More than 18,000 comments were received by CDPH regarding the proposed regulation. The proposed final regulation documents include the Summary and Response to comments received.

The proposed final regulation will take effect after it has been reviewed and approved by OAL in compliance with the Administrative Procedures Act. This review can take up to 30 working days to complete. Once approved, the regulation is then filed with the Secretary of State and will become effective the first day of the following quarter.

"The drinking water standard for hexavalent chromium of 10 parts per billion will protect public health while taking into consideration economic and technical feasibility as required by law," said Dr. Ron Chapman, CDPH director and state health officer.

If the regulation is approved as expected, implementation of the new drinking water standard for hexavalent chromium will begin July 1, 2014.

Today's filing also complies with timelines imposed by the Alameda Superior Court in *Natural Resources Defense Council, Inc. v. California Department of Public Health.*

The department's submission to OAL can be found on the CDPH website.

www.cdph.ca.gov

Bohn, Brent

From: Sasso, Alan
Sent: Thursday, August 22, 2013 3:06 PM
To: Elaine.Khan@oehha.ca.gov; Gibbons, Catherine
Subject: RE: CA Cr6 MCL

Thanks Elaine,

Also, we've recently posted new information regarding the hexavalent chromium webinar to our website:

http://www.epa.gov/iris/irisworkshops/cr6/index.htm

The panelist names, and the white paper are now public.

Hope you can call in, despite the early time! We have a panelist in Italy, which is why the time is so early.

Be sure to register if you haven't already. Let us know if you have any questions or need any clarifications on the new information posted.

Thanks again,

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
(703)-347-0179

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Thursday, August 22, 2013 2:53 PM
To: Gibbons, Catherine
Cc: Sasso, Alan
Subject: CA Cr6 MCL

Hi, Catherine and Alan.

Fyi, California is releasing a proposed Cr6 MCL (10 ppb) for public comment.
http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6.aspx

Elaine

Bohn, Brent

From: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Sent: Thursday, August 22, 2013 4:22 PM
To: Sasso, Alan; Gibbons, Catherine
Subject: RE: CA Cr6 MCL

Thanks, Alan. I'm registered and looking forward to the workshop!

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Thursday, August 22, 2013 12:06 PM
To: Khan, Elaine@OEHHA; Gibbons, Catherine
Subject: RE: CA Cr6 MCL

Thanks Elaine,

Also, we've recently posted new information regarding the hexavalent chromium webinar to our website:

http://www.epa.gov/iris/irisworkshops/cr6/index.htm

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Be sure to register if you haven't already. Let us know if you have any questions or need any clarifications on the new information posted.

Thanks again,

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
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U.S. Environmental Protection Agency
(703)-347-0179

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Thursday, August 22, 2013 2:53 PM
To: Gibbons, Catherine
Cc: Sasso, Alan
Subject: CA Cr6 MCL

Hi, Catherine and Alan.